

1 **Prognostic Value of Serum/Plasma Neurofilament Light Chain for COVID-19 Associated**
2 **Mortality**

3 Raturaj R. Masvekar¹, Peter Kosa¹, Kimberly Jin¹, Kerry Dobbs¹, Michael A. Stack¹, Riccardo
4 Castagnoli¹, Virginia Quaresima², Helen C. Su¹, Luisa Imberti², Luigi D. Notarangelo¹, and
5 Bibiana Bielekova^{1*}

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7 **Affiliations**

8 ¹National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda,
9 MD.

10 ²CREA Laboratory (AIL Center for Hemato-Oncologic Research), Diagnostic Department,
11 ASST Spedali Civili di Brescia, Brescia, Italy.

12 *To whom correspondence should be addressed: Bibiana Bielekova, MD, Neuroimmunological
13 Diseases Section (NDS), National Institute of Allergy and Infectious Diseases (NIAID), National
14 Institutes of Health (NIH), Building 10, Room 5N248, 10 Center Drive, MSC1444, Bethesda,
15 Maryland 20892, USA. (Bibi.Bielekova@nih.gov).

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33 **ABSTRACT**

34 Given the continued spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),
35 early predictors of coronavirus disease 19 (COVID-19) mortality might improve patients'
36 outcomes. Increased levels of circulating neurofilament light chain (NfL), a biomarker of neuro-
37 axonal injury, have been observed in patients with severe COVID-19. We investigated whether
38 NfL provides non-redundant clinical value to previously identified predictors of COVID-19
39 mortality.

40 We measured serum or plasma NfL concentrations in a blinded fashion in 3 cohorts totaling 338
41 COVID-19 patients. In cohort 1, we found significantly elevated NfL levels only in critically ill
42 COVID-19 patients compared to healthy controls. Longitudinal cohort 2 data showed that NfL is
43 elevated late in the course of the disease, following two other prognostic markers of COVID-19:
44 decrease in absolute lymphocyte count (ALC) and increase in lactate dehydrogenase (LDH).
45 Significant correlations between LDH and ALC abnormalities and subsequent rise of NfL
46 implicate multi-organ failure as a likely cause of neuronal injury at the later stages of COVID-
47 19. Addition of NfL to age and gender in cohort 1 significantly improved the accuracy of
48 mortality prediction and these improvements were validated in cohorts 2 and 3.

49 In conclusion, although substantial increase in serum/plasma NfL reproducibly enhances
50 COVID-19 mortality prediction, NfL has clinically meaningful prognostic value only close to
51 death, which may be too late to alter medical management. When combined with other
52 prognostic biomarkers, rising longitudinal NfL measurements triggered by LDH and ALC
53 abnormalities would identify patients at risk of COVID-19 associated mortality who might still
54 benefit from escalated care.

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68 INTRODUCTION

69 Since early 2020, the COVID-19 pandemic has exhausted medical systems worldwide. Even
70 after the development of safe and effective vaccines, SARS-CoV-2 continues to spread (COVID
71 Live Update - Worldometer). A reliable early predictor of COVID-19 associated mortality would
72 help prioritize use of medical resources and maximize patient survival.

73 Neurofilaments are essential cytoskeleton proteins of the central and peripheral axons exclusive
74 to the nervous system. Of three neurofilament subunits, neurofilament light chain (NfL) has the
75 lowest molecular weight and easily diffuses from parenchyma to CSF and blood (Fuchs and
76 Cleveland, 1998; Scherling et al., 2014; Alirezaei et al., 2020). Recent developments of
77 ultrasensitive assays, such as Single Molecule Array (SIMOA), allow reproducible measurement
78 of low NfL concentrations in serum or plasma (Rissin et al., 2010; Kan et al., 2012).
79 Consequently, blood NfL became a key noninvasive biomarker of acute neuronal injury in
80 diverse neuropathological conditions (Barro et al., 2020).

81 Although previous studies have demonstrated association between COVID-19 morbidity and
82 CNS damage (Aamodt et al., 2020; Ameres et al., 2020; Kanberg et al., 2020, 2021; Prudencio et
83 al., 2021), several questions still remain unanswered: 1) Does a single measurement of NfL
84 provide meaningful prognostic information at individual patient level?; 2) Is there a relationship
85 between NfL and previously described COVID-19-associated mortality biomarkers (Yan et al.,
86 2020) of prognostic value, such as ALC, C-reactive protein [CRP] and LDH?; and 3) Does NfL
87 improve COVID-19 mortality prediction by demographic markers such as age and gender?

88

89 MATERIALS and METHODS

90 Research subjects and cohorts

91 Serum or plasma samples from COVID-19 patients admitted at ASST Spedali Civili (Brescia,
92 Italy) were obtained through Laboratory of Clinical Immunology and Microbiology (LCIM),
93 National Institute of Allergy and Infectious Diseases (NIAID), under Institutional Review Board
94 (IRB)-approved protocols (Comitato Etico Provinciale: NP 4000 - Studio CORONALab and NP
95 4408 - Studio CORONALab and ClinicalTrials.gov: NCT04582903). SARS-CoV-2 infection was
96 confirmed using nasopharyngeal swab – polymerase chain reaction (PCR) test. COVID-19
97 disease severity was determined as per Diagnosis and Treatment Protocol for Novel Coronavirus
98 Pneumonia guidelines, released by the National Health Commission & State Administration of
99 Traditional Chinese Medicine (Wei PF, 2020). Serum and plasma samples from healthy controls
100 (HC) and multiple sclerosis (MS) subjects were collected at Neuroimmunological Diseases
101 Section (NDS), NIAID after informed consent under IRB-approved protocol (ClinicalTrials.gov:
102 NCT00794352). The NfL levels measured in HC and MS subgroups were previously reported
103 (Masvekar R et al., 2021) and are used in the current study only as a positive control of neuronal
104 injury; the measurements of other COVID-19 prognostic biomarkers in these control samples
105 were not reported previously.

106 378 serum or plasma samples were collected from 338 COVID-19 patients grouped into 3
107 independent cohorts (Figure 1, Table 1, and Supplementary Data File 1). In cohort 1, 30 cross-
108 sectional samples were collected from COVID-19 patients with 3 levels of disease severity. In

109 cohort 2, 60 longitudinal samples were collected from 20 critically ill COVID-19 patients (T1,
110 T2, and T3: collected averagely at 5 to 10 day intervals, within 30 days of hospitalization).
111 Cohort 3 consisted of 288 cross-sectional samples collected from critically ill COVID-19
112 patients where a large proportion of the subjects eventually died (39.2%).

113 **NfL single molecular array (Simoa™) assay**

114 NfL concentrations in serum or plasma samples were measured using Simoa™ assay (Catalog #
115 103186; Quanterix, Billerica, MA, USA). Samples were diluted 1:4 and randomly distributed on
116 96-well plates. Quality control (QC) samples provided with the kit had concentrations within the
117 pre-defined range and the coefficient of variance (CV) across the plates was < 10%. All samples
118 were analyzed blindly under alpha-numeric codes. The diagnostic codes were broken only after
119 QC verified NfL concentrations were reported to the database manager.

120 **Adjustment for effect of healthy aging**

121 As serum/plasma NfL levels increase with physiological aging (Disanto et al., 2017), the
122 measured NfL concentrations were adjusted for effect of healthy aging as described previously
123 (Masvekar R et al., 2021). Following age vs serum- or plasma-NfL equations from HC cohorts
124 were used: $\ln(\text{serum NfL}) = 0.0177 * \text{Age} + 0.9696$ and $\ln(\text{plasma NfL}) = 0.0158 * \text{Age} + 1.247$.
125 The age-adjusted NfL concentrations represent residuals from the above-stated linear regression
126 models.

127 **Statistical analyses**

128 NfL levels were compared across disease diagnosis and severity subgroups using either Kruskal-
129 Wallis ANOVA or Welch's t-test. Correlations between NfL and systemic markers of COVID-
130 19 morbidity were evaluated using Spearman analysis and linear regression model.

131 Prediction models of COVID-19 associated mortality were developed in R Studio Version
132 1.1.463 (R version 4.0.2) using logistic regression (*glm* function of the "stat" package) (R: The R
133 Project for Statistical Computing). Optimal cutoff for the predictive models was calculated using
134 *optimalCutoff* function of the "InformationValue" package ([https://cran.r-](https://cran.r-project.org/web/packages/InformationValue/index.html)
135 [project.org/web/packages/InformationValue/index.html](https://cran.r-project.org/web/packages/InformationValue/index.html)). The receiver operating characteristic
136 curve (ROC) was calculated using *roc* function of the "pROC" package (Robin et al., 2011).

137

138 **RESULTS**

139 **NfL levels increase with COVID-19 severity and mortality**

140 Although increased blood NfL levels have been reported in patients with severe COVID-19
141 (Aamodt et al., 2020; Ameres et al., 2020; Kanberg et al., 2020, 2021; Prudencio et al., 2021),
142 previous studies had insufficient numbers of subjects who died from the disease to assess
143 whether NfL can predict COVID-19 mortality.

144 To fill this knowledge gap, we measured NfL levels in 30 COVID-19 patients with 3 levels of
145 severity: 1) moderate severity (n = 10); 2) critical condition but survived (n = 10); and 3) critical
146 condition but died (n = 10). Positive and negative control subgroups consisted of 1) patients with
147 acute COVID-19-like symptoms admitted in critical health conditions who tested negative for

148 SARS-CoV-2 infection (n = 10); 2) HC (n = 58); 3) MS patients with acute focal CNS
149 inflammation measured as contrast-enhancing lesions on brain MRI (active MS, n = 35); and 4)
150 MS patients without evidence of acute focal CNS inflammation (non-active, n = 35).

151 After diagnostic codes were unblinded, we found elevated levels of NfL in COVID-19 patients
152 compared to HC (Figure 2A). NfL levels in COVID-19 patients increased with disease severity,
153 but only cohorts of critically ill COVID-19 and MS patients reached statistical significance
154 compared to HC.

155 Next, we compared cohort differences in other blood biomarkers of COVID-19 morbidity: ALC,
156 CRP, and LDH (Figures 2B, 2C and 2D). Like NfL, decreased ALC and increased LDH
157 correlated with COVID-19 severity; statistically significant differences in ALC and LDH were
158 observed only in critically ill COVID-19 patients compared to HC. Interestingly, although non-
159 COVID-19 acute respiratory illness control had levels of COVID-19 prognostic biomarkers (i.e.,
160 NfL, ALC, and LDH) comparable to HC, they had the highest CRP levels.

161 We conclude that NfL, LDH, and ALC abnormalities increase with COVID-19 severity, are
162 associated with COVID-19 mortality, and can differentiate COVID-19 from other acute
163 respiratory conditions that lead to ICU admission.

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165 **In COVID-19 patients NfL rises close to death, trailing transient abnormalities in ALC and** 166 **LDH by 5 to 20 days**

167 The earlier a biomarker can identify patients at risk for COVID-19 mortality, the greater its
168 clinical value. Because none of the previous studies addressed the dynamics of NfL rise in
169 COVID-19 and compared it to the dynamics of other prognostic biomarkers, we addressed this
170 knowledge gap in the longitudinal cohort 2.

171 We measured NfL in 60 samples collected from 20 critically ill COVID-19 patients within 30
172 days of hospitalization, at three timepoints (T1, T2 and T3) taken at approximately 5 to 10 day
173 intervals. We observed statistically significant, progressive increases (T1 vs. T2 and T3) in NfL
174 levels only in patients who later died (Figure 3A).

175 When plotting measurements against day of hospitalization, the greatest rise in NfL occurred
176 close to death (Figure 3B and Supplementary Figure 1). Consistent with prior reports that NfL
177 levels remain elevated for weeks (up to 3 months) following acute CNS injury (Thelin et al.,
178 2017), increased NfL in COVID-19 patients did not return to normal within the observation
179 period. In contrast, ALC, LDH, and CRP (Supplementary Figure 1) demonstrated large day-to-
180 day fluctuations and were also frequently elevated in surviving patients (Supplementary Figure
181 2).

182 To assess if transient abnormalities in LDH, CRP, and ALC levels precede increases in NfL, we
183 investigated correlations between these systemic markers measured at initial time-points (T1 and
184 T2), with NfL measured later (i.e., T1 vs T2, T1 vs T3 and T2 vs T3). Only 3 of these
185 comparisons reached statistical significance (Figure 3C), with the strongest relationship observed
186 between LDH measured at first time point (T1) and NfL measured at last time point (T3), which

187 explains almost 60% of variance ($R^2 = 0.598$, $p = 0.0001$). Consistent with the lack of
188 association of CRP measurements with COVID-19 severity, CRP elevations did not predict
189 subsequent rise in NfL.

190 We conclude that critically ill COVID-19 patients experience earlier abnormalities in ALC and
191 LDH measurements, which are strongly associated with later elevation in NfL levels. While
192 these critically high NfL levels predict COVID-19 mortality, they peak shortly before death,
193 which may be too late to alter medical management.

194

195 **NfL measured close to death enhances mortality prediction of age and gender-based** 196 **classifier**

197 As all the above-described observations supported clinical value of NfL to predict COVID-19
198 mortality, we sought to quantify this predictive value on an individual patient level and compare
199 it to demographic prognostic markers such as age, gender, and comorbidities.

200 In the cohort 1, used as a training cohort, we predicted COVID-19 mortality using measured NfL
201 as a continuous variable (Figure 4A, left panel). Single, cross-sectional NfL measurements could
202 not reliably predict death, reaching an area under receive operator characteristic curve (AUROC)
203 of only 0.61 with 95% confidence interval ([CI]: 0.33-0.89) crossing the value of random
204 guessing (i.e., AUROC 0.5). The optimal cut-off from NfL to predict mortality from cohort 1
205 ROC curve was 124 pg/ml.

206 As shown in Table 1, cohorts 1 and 2 were not matched for demographic predictors of COVID-
207 19 mortality: in both cohorts, patients who survived were generally younger, with higher
208 proportion of females and lower proportion of subjects with comorbidities. Therefore, it should
209 not be surprising that NfL measurements alone, ignoring these important demographic variables,
210 had low predictive power. Instead, we built a prognostic classifier that integrated NfL
211 (dichotomized based on optimal cut-off 124 pg/ml) with age and gender, and compared it to the
212 model(s) without NfL. We also tested a more complex classifier consisting of dichotomized NfL,
213 age, gender, and comorbidities, but observed weaker independent validation of this model
214 compared to a model without comorbidities (Supplementary Figure 3). For the sake of space and
215 clarity we will present data only on the strongest model.

216 Adding dichotomized NfL enhanced predictive value of age and gender in cohort 1 from
217 AUROC 0.8 to 0.85 and p-value from 0.023 to 0.0068 (Figure 4, cohort 1 panel).

218 Next, we sought to assess performance of the leading mortality predictor in Cohort 2, which did
219 not contribute to model generation (Figure 4B). Addition of dichotomized NfL to the age and
220 gender at first longitudinal time-point (T1) did not improve predictive value of the model,
221 consistent with observation that at early timepoint the NfL values were indistinguishable
222 between patients who survived and those who died. In contrast, NfL significantly improved the
223 predictive power of the combined classifier at later time-points (T2 and T3; T2: AUROC from
224 0.76 (CI: 0.53-0.99) to 0.89 (CI: 0.74-1.00) and p-value from 0.06 to 0.0048; T3: AUROC from
225 0.76 (0.53-0.99) to 0.96 (0.87-1.00) and p-value from 0.06 to 0.00094).

226 We conclude that NfL measurement provides additive COVID-19 mortality predictive value to
227 the traditional demographic prognostic factors, provided that NfL is measured in critically ill
228 patients later in disease.

229 Finally, we were able to assess the non-redundant prognostic value of NfL in a unique large
230 cohort of patients with high COVID-19 mortality risk (i.e., elderly patients with high proportion
231 of males with comorbidities; Figure 4C). As expected, out of these 288 critically ill COVID-19
232 patients, a large proportion (n=113; 39.2%) eventually died.

233 Although surviving and dying cohorts were matched for age, gender, and comorbidities as
234 univariate predictors (Table 1), the combined age plus gender model correctly predicted a
235 marginally higher mortality in the cohort of subjects who eventually died (10% vs 93%;
236 $p=0.047$). NfL levels differentiated survivors from non-survivors with much stronger statistical
237 significance ($p = 4.1e-08$). Adding dichotomized NfL to demographic data improved the
238 accuracy of mortality prediction compared to demographic data alone. Specifically, the AUROC
239 increased from 0.57 (CI: 0.50-0.64) to 0.62 (CI: 0.55-0.69) and p-value improved from 0.047 to
240 0.00063. Nevertheless, the sensitivity (71.4%) and specificity (40.7%) of this predictor remained
241 weak in this unique cohort.

242

243 **DISCUSSION**

244 This study validates reports linking high serum/plasma NfL levels to COVID-19 severity
245 (Aamodt et al., 2020; Ameres et al., 2020; Kanberg et al., 2020, 2021; Prudencio et al., 2021;
246 Sutter et al., 2021). Our longitudinal measurements demonstrated that rise in NfL generally
247 occurs during hospitalizations of critically ill patients and trails other transient laboratory
248 abnormalities such as decreased ALC and increased LDH by 5 to 20 days. The degree of LDH
249 increase is a strong determinant of subsequent magnitude of NfL rise, suggesting that COVID-
250 19-associated CNS injury is secondary to damage of other critical organs, such as liver, kidneys,
251 and lungs. This conclusion aligns with pathology studies ruling out strong primary infiltration of
252 CNS tissue by the SARS-CoV-2 or by immune system; those studies instead attribute COVID-19
253 associated CNS damage to processes such as hypoxia or intravascular coagulation (Serrano et al.,
254 2021).

255 Compared to previous studies of NfL in COVID-19, we studied a cohort of patients in which a
256 high proportion eventually died ($133/338 = 39.3\%$). This allowed us to unequivocally link high
257 serum/plasma NfL levels also with COVID-19 mortality, something that remained ambiguous in
258 the previous studies.

259 We constructed a model that combined demographic predictors of COVID-19 mortality with
260 NfL measurement and validated its greater predictive accuracy. Nevertheless, the accuracy of
261 this classifier varied between the cohorts, depending on the timing of NfL measurement (i.e.,
262 later measurements enhanced predictive power) and underlying premorbid risk. Indeed,
263 comparing model performance among our 3 cohorts, it appeared that NfL has greater predictive
264 value in younger (cohorts 1 and 2) versus older (cohort 3) subjects. This is perhaps not surprising
265 as younger patients with fewer comorbidities have higher likelihood of withstanding multi-organ
266 failure and therefore CNS injury may become key determinant of their survival. In contrast,

267 elderly subjects with high premorbid risk rapidly succumb to multi-organ failure before CNS
268 injury manifests clinically or by high NfL concentrations.

269 Integrating all our observations, we recommend that NfL should be measured longitudinally and
270 integrated with existing prognostic markers to optimize care. For example, a screening NfL
271 measurement at the beginning of hospitalization, expected to be normal in most patients, might
272 identify a few subjects with either neurological comorbidity or with advanced stage of COVID-
273 19 who require care focused on preventing further CNS injury. After an initial negative NfL test,
274 critically ill COVID-19 patients might be best monitored by standard laboratory tests such as
275 LDH and ALC. Identified spikes should prompt more aggressive management that includes
276 longitudinal NfL monitoring approximately every 5 days. Any increase in NfL should be
277 considered a poor prognostic indicator necessitating escalation therapies. These may include
278 neuro-protective strategies that lower CNS metabolism, such as systemic cooling or barbiturates.
279 Stabilization of NfL levels indicates that escalation therapy worked, while further increases
280 signify continuous neuro-axonal injury that must be stopped to limit mortality.

281 While the COVID-19 pandemic demonstrated prognostic value of NfL in critically ill patients
282 with SARS-CoV-2 infection, non-invasive, ultrasensitive measurement of NfL could be used to
283 monitor neuronal injury in all comatose, or heavily sedated critically ill patients regardless of
284 SARS-CoV-2 infection status. Ultra-sensitive assays will hopefully become broadly adopted by
285 clinical laboratories and might include in the future other CNS-derived analytes for enhanced
286 accuracy of non-invasive monitoring of CNS tissue.

287

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292 providing us COVID-19 patients’ serum/plasma samples.

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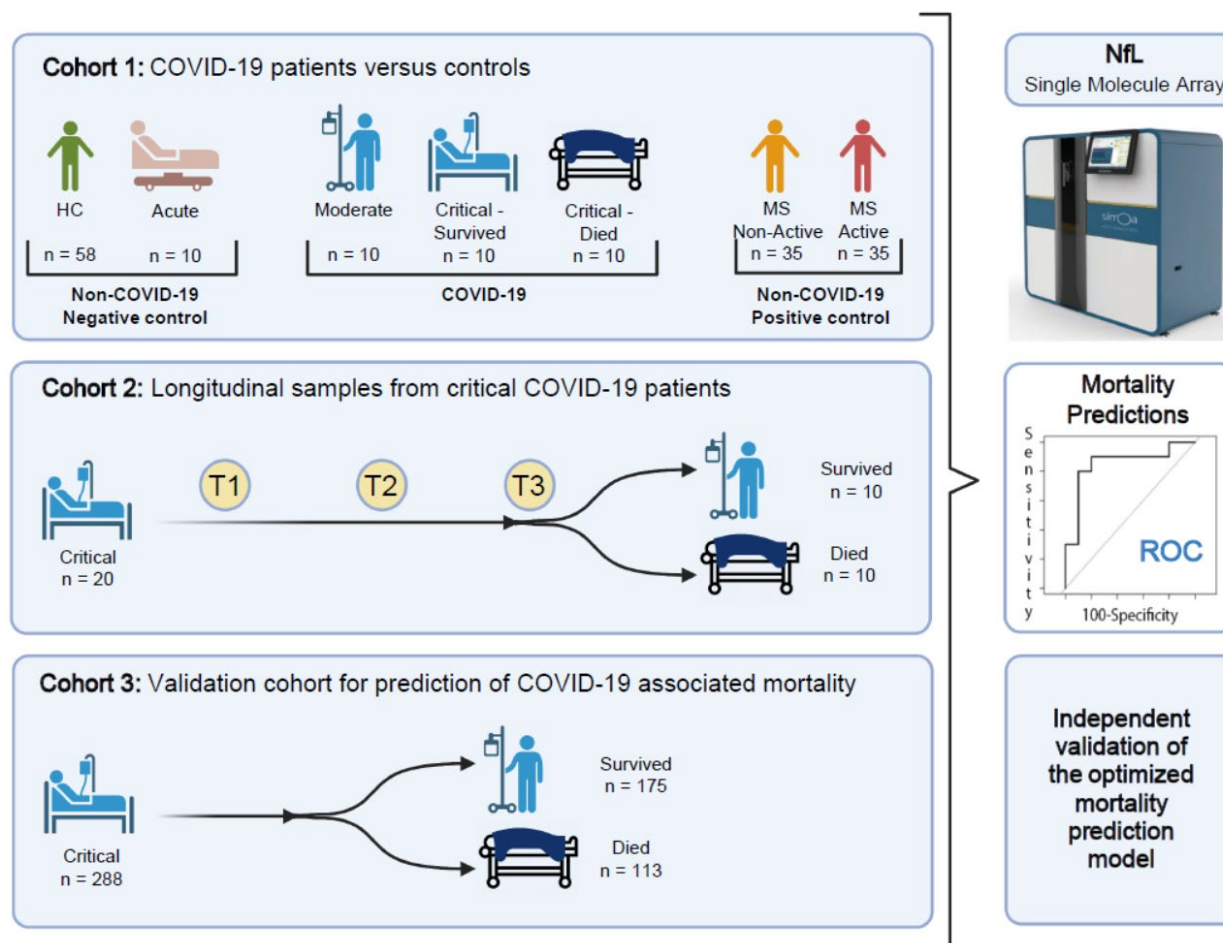
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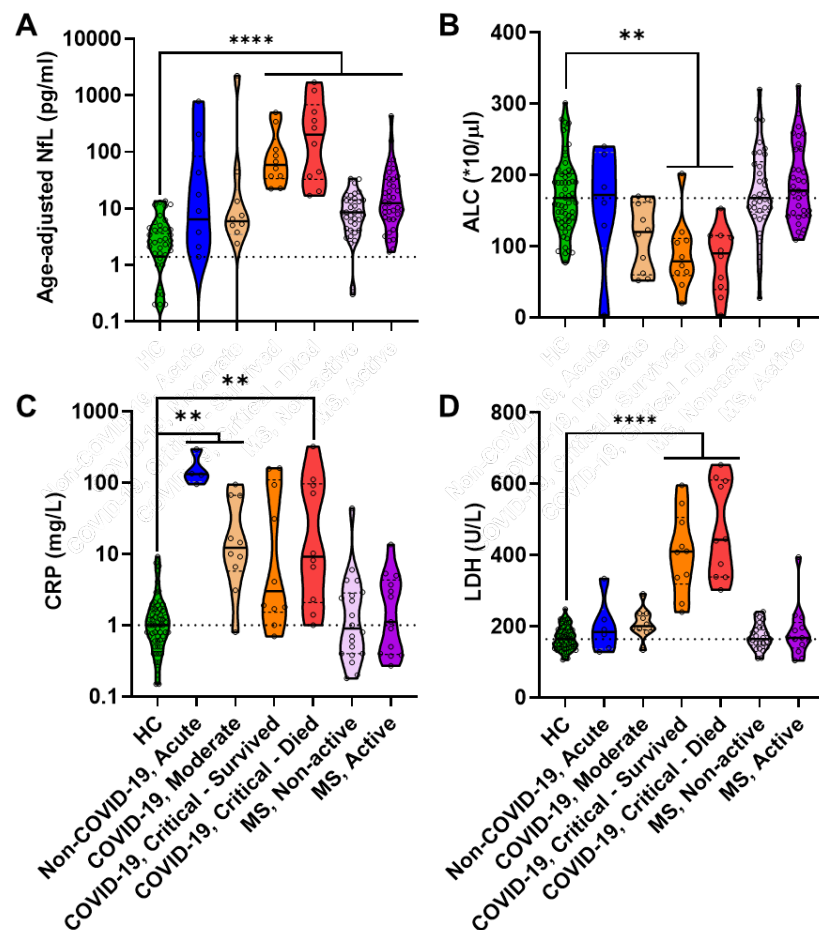
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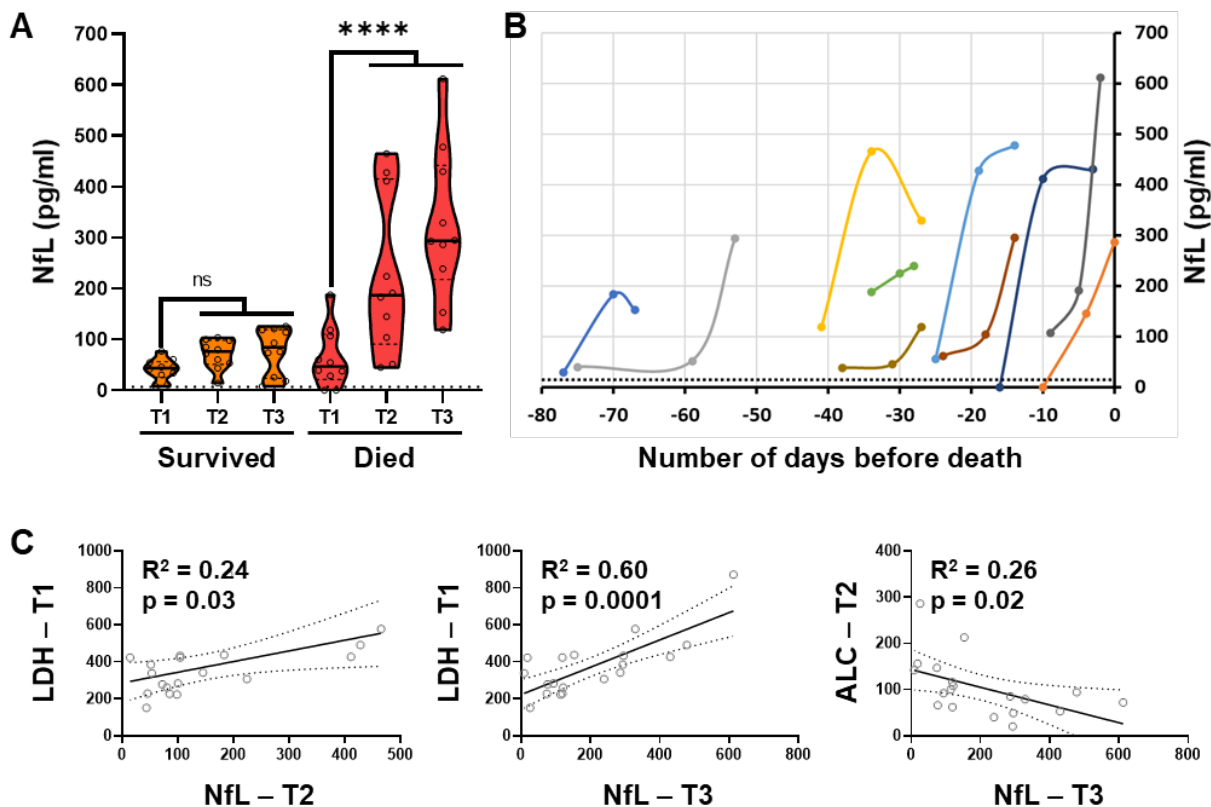
353 **FIGURES**



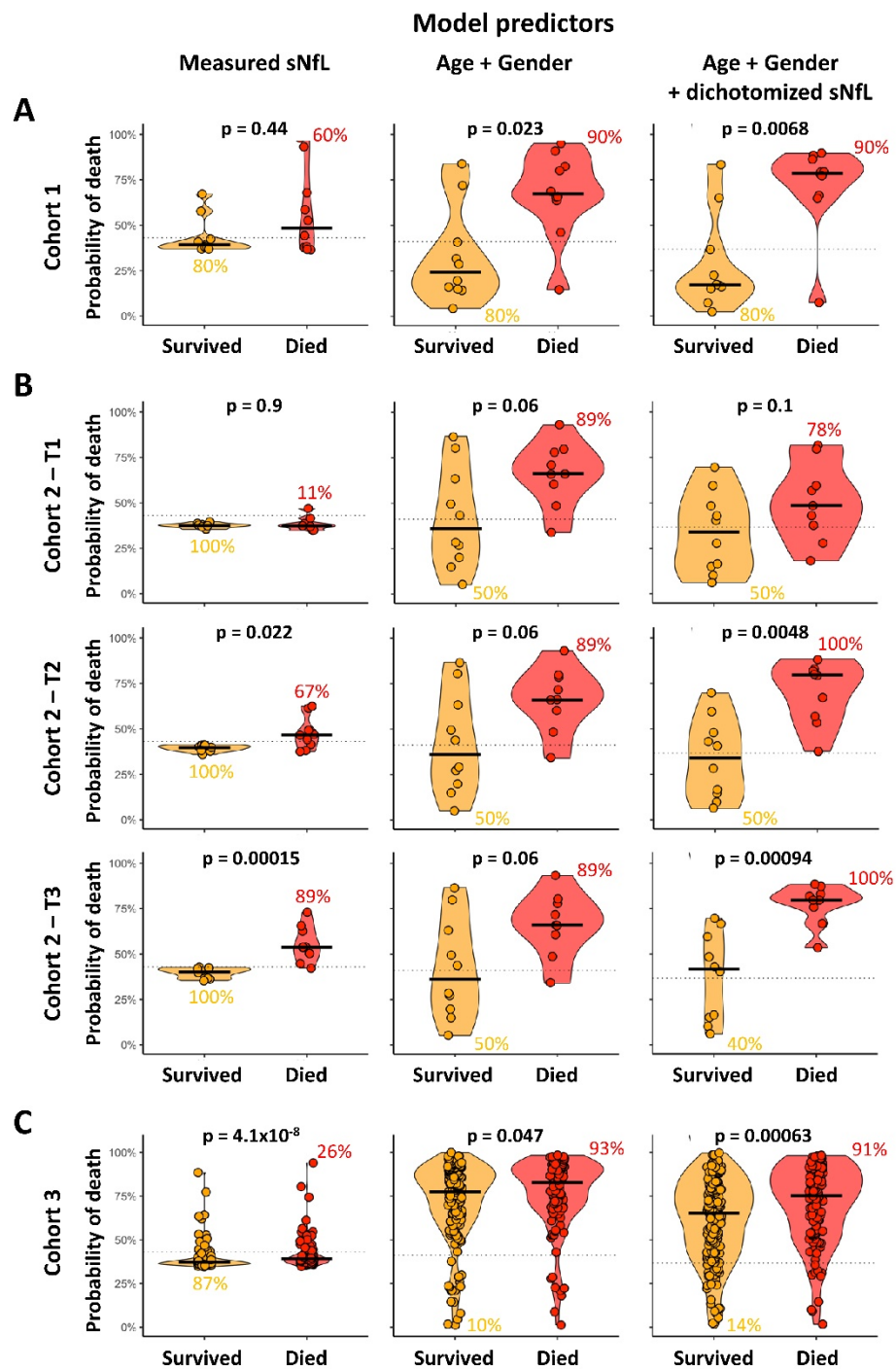
355 **Figure 1:** Patient selection, objectives, and experiment outlines of 3 independent cohorts. Cohort
 356 1 aims to analyze NfL cross-sectionally across disease diagnosis and severity categories. In
 357 cohort 2, objective was to analyze NfL levels in critically ill COVID-19 patients, longitudinally
 358 at 3 different time-points (T1, T2, and T3: collected averagely at 5 to 10 days interval, within 30
 359 days of hospitalization). Observed additional prognostic value of NfL with traditional
 360 demographic factors (age and gender) from cohorts 1 and 2, was independently validated in
 361 cohort 3.



363 **Figure 2:** In cohort 1, (A) NfL, (B) ALC, (C) CRP, and (D) LDH were compared across HC vs.
364 COVID-19 disease severity and multiple sclerosis disease activity subgroups using Kruskal-
365 Wallis ANOVA; **p < 0.005 and ****p < 0.0001. The dotted line on each plot indicates the
366 median of HC.



368 **Figure 3:** In cohort 2, (A) NfL levels at 3 different time points (T1, T2, and T3: collected on
 369 average at 5 to 10 day intervals, within 30 days of hospitalization) in critically ill COVID-19
 370 patients were compared (survived versus died) using Kruskal-Wallis ANOVA; **** $p < 0.0001$.
 371 The dotted line indicates the median of the HC. (B) Longitudinal NfL levels in critical COVID-
 372 19 patients who died, plotted with respect to number of days before death. Each line represents
 373 data from an individual patient. The dotted line represents upper limit in HC (i.e., mean + 3*SD
 374 = 20 pg/ml). (C) Correlations between systemic biomarkers' measurements at earlier time points
 375 (T1 and 2) and NfL measurements at later time points (T2 and T3) were assessed using linear
 376 regression analysis. R^2 and p-value are represented on respective correlation plots. The dotted
 377 line indicates 95% confidence interval.



379 **Figure 4:** Comparisons of 3 predictive models of COVID-19 associated mortality: continuous
 380 NfL measurement, Age plus Gender, and Age plus Gender plus dichotomized NfL in 3
 381 independent cohorts; (A) cohort 1, (B) cohort 2, and (C) cohort 3. The dotted line on each plot
 382 represents the optimal cut-off for respective model predictor. The numerical values beside
 383 respective subgroups (Survived or Died) on each plot represents the percentage of correctly
 384 classified patients.

385

386 **TABLE**

			Non-COVID-19				COVID-19		
			Negative Control		Positive Control		Critical		
			HC	Acute	MS, Non-Active	MS, Active	Moderate	Survived	Died
	n		58	10	35	35	10	10	10
Cohort 1 (N = 168)	Age (years)	mean (SD)	41.1 (13.7)	43.6 (20.8)	52.8 (11.3)*	37.6 (10.8)	51.8 (13.9)	56.1 (11.4)*	69.4 (11.7)*
	Gender	female (%)	28 (48.3)	2 (20.0)	16 (45.7)	22 (62.9)	3 (30.0)	2 (20.0)	1 (10.0)*
	Comorbidities	yes (%)	NA	7 (70.0)	NA	NA	8 (80.0)	7 (70.0)	9 (90.0)
	n		18	-	-	-	-	10	10
Cohort 2 (N = 38)	Age (years)	mean (SD)	44.1 (10.5)	-	-	-	-	64.7 (9.9)*	67.0 (6.9)*
	Gender	female (%)	9 (50.0)	-	-	-	-	5 (50.0)	0 (0.0)*#
	Comorbidities	yes (%)	NA	-	-	-	-	6 (60.0)	10 (100.0)#
	n		-	-	-	-	175	113	
Cohort 3 (N = 288)	Age (years)	mean (SD)	-	-	-	-	-	73.8 (10.7)	77.3 (10.4)#
	Gender	female (%)	-	-	-	-	-	40 (22.8)	35 (30.9)
	Comorbidities	yes (%)	-	-	-	-	-	123 (70.3)	87 (76.9)

388 **Table 1:** Demographic details of the 3 cohorts. Age (ANOVA or unpaired t-test), gender, and
 389 comorbidities distribution) were compared across disease diagnosis and severity subgroups using
 390 Chi-square test. *p < 0.05 vs HC and #p < 0.05 vs COVID-19, Critical - Survived.

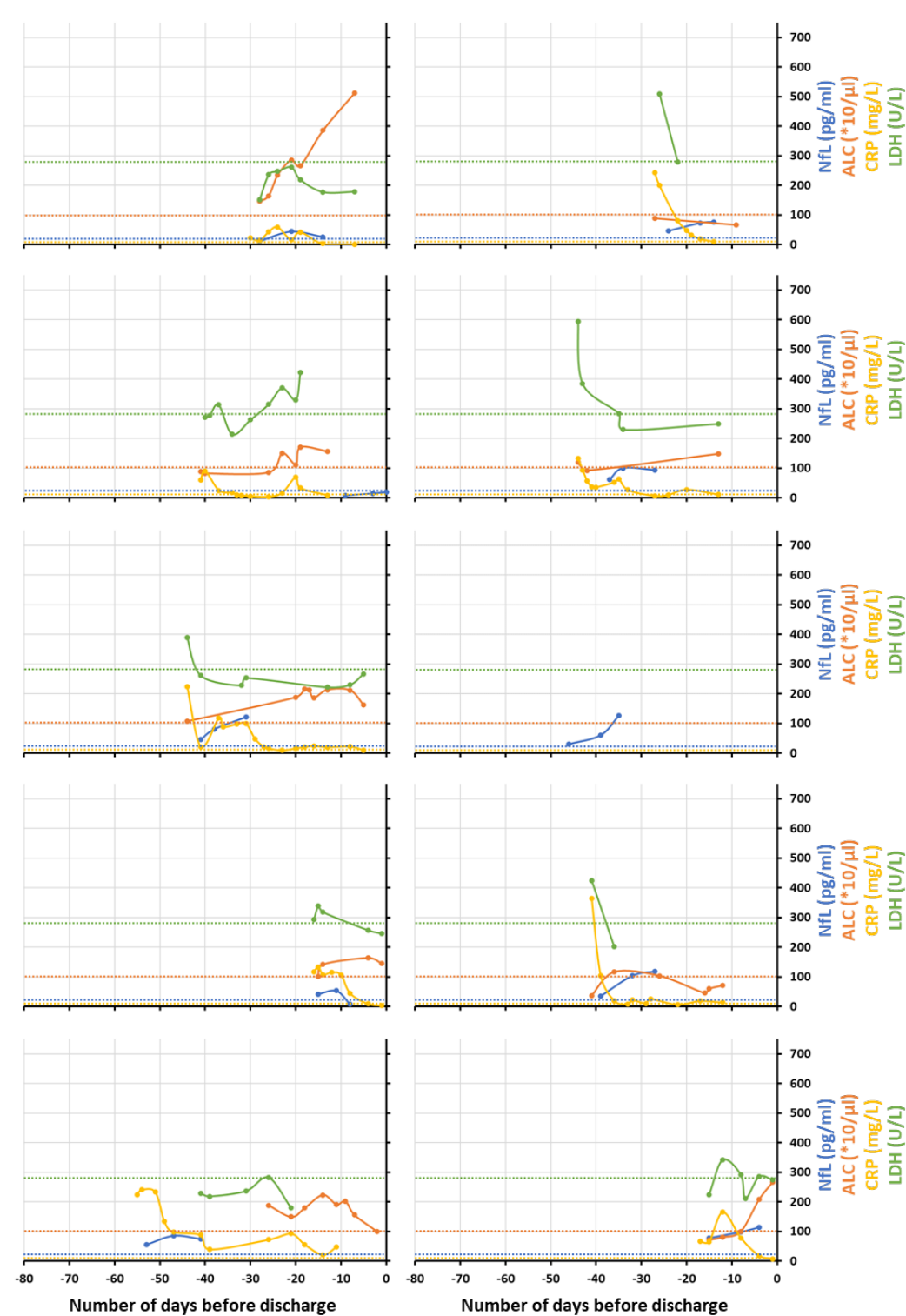
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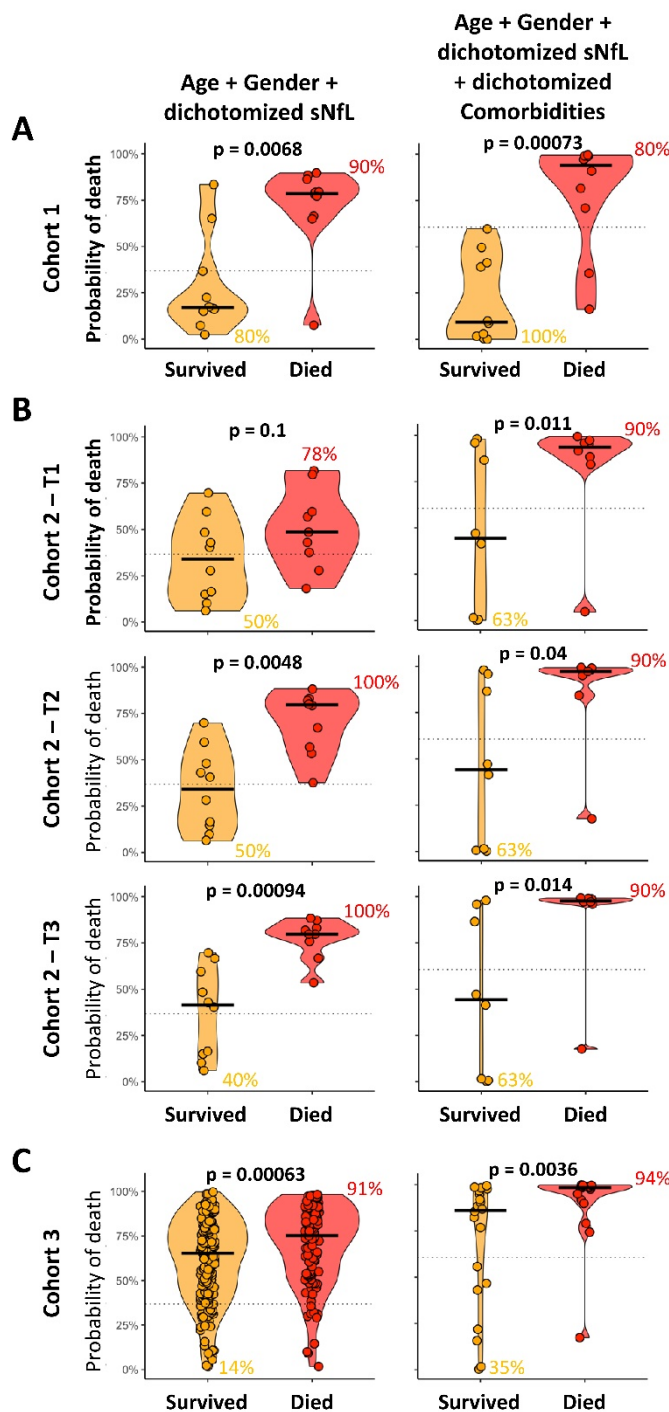
393 SUPPLEMENTARY FIGURES



395 **Supplementary Figure 1:** In cohort 2, longitudinal NfL (blue), ALC (orange), CRP (yellow)
396 and LDH (green) levels in critically ill COVID-19 patients those who died, plotted with respect
397 to number of days before death. Each plot represents an individual patient data. The respective
398 color dotted lines represent upper (for NfL, CRP and LDH) or lower (for ALC) limit for HC for
399 respective biomarker (NfL: 20 pg/ml, ALC: 100 *10/ μ l, CRP: 5 mg/L and LDH: 280 U/L).



401 **Supplementary Figure 2:** In cohort 2, longitudinal NfL (blue), ALC (orange), CRP (yellow)
402 and LDH (green) levels in critically ill COVID-19 patients those who survived, plotted with
403 respect to number of days before discharge. Each plot represents an individual patient data. The
404 respective color dotted lines represent upper or lower limit for HC for respective biomarker.



406 **Supplementary Figure 3:** Comparisons of 2 predictive models of COVID-19 associated
 407 mortality: Age plus Gender plus dichotomized NfL and Age plus Gender plus dichotomized NfL
 408 plus dichotomized comorbidities in 3 independent cohorts; (A) cohort 1, (B) cohort 2, and (C)
 409 cohort 3. The dotted line on each plot represents the optimal cut-off for respective model
 410 predictor. The numerical values beside respective subgroups (Survived or Died) on each plot
 411 represents the percentage of correctly classified patients.

412

413 **SUPPLEMENTARY DATA FILE**

414 **Supplementary data file 1:** Cohort, demographics, disease and severity diagnosis, timeline of
415 important events during disease, NfL – raw and HC age-adjusted measurements, comorbidities
416 and lab test measurements for systemic markers (ALC, CRP and LDH) data for all subjects (HC
417 = 76, Non-COVID-19 Acute = 10, MS Non-active = 35, MS Active = 35, COVID-19: moderate
418 = 10, critical - survived = 195, and critical - deceased = 133). Patients were recoded and
419 personally identifiable information were excluded.